studies was that it was still not possible to conclude with certainty that Claude's large particles constituted mitochondria. A major reason was that when examined microscopically, the particles in the fraction did not exhibit the rod-shaped appearance of mitochondria and did not respond to the usual mitochondrial stains. One of the main strategies of the new team of researchers was to vary the media used for cell fractionation. Although Claude had tried several variations in fractionation techniques, he had continued to use either distilled water or saline solutions as the media. Hogeboom, Schneider, and Palade (1948) explored sucrose as well as other sugar solutions. With approximately isotonic (0.25 *M*) sucrose, they noted that the large granules did not agglutinate and were roughly of the same size as with isotonic saline. The particles, however, did not retain the elongated shape of mitochondria. When they tried more hypertonic sucrose solutions, the particles became more elongated, with the percentage of rod-like shapes reaching a maximum with 0.8 to 1.0 *M* sucrose solutions. The researchers therefore adopted 0.88 *M* sucrose solutions for their research.

Not only did the large granules now more closely resemble mitochondria as observed in whole cells, they also stained with Janus green B, a stain selective for mitochondria. The researchers also dispelled Claude's claim that the large granule fraction was partially or even largely comprised of secretory granules. Secretory granules stain with neutral red and once mitochondria were made to retain their rod-like shape, the two could be distinguished. The secretory granules appeared to break up after the rupture of the cell membrane and to migrate centripetally; they were thus not part of the large granule fraction. Having developed a fraction that was demonstrably mitochondrial, the researchers replicated the findings of the earlier study that this fraction housed the succinoxidase system. They now said, more boldly than in 1946, "The mitochondrion can therefore be considered as a complicated functional unit possessing two of the most important respiratory enzyme systems of the cell . . ." (Hogeboom et al., 1948, p. 360).

With the improved fractionation procedures, Hogeboom and his colleagues continued the quantitative analysis of the composition of the different fractions. In addition to confirming that the mitochondria contained nearly all the succinoxidase, they established that the microsome fraction contained about 50% of the pentose nucleic acid. From his earliest studies of the small particle fraction, Claude had noted these particles were high in ribose nucleic acid (RNA). That, however, seemed to be the only clue to their function, and so

0.1 μ in diameter that he and Fullam had identified in mitochondria in their electron microscope studies (see below).